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Withanolides from three species of the genus *Deprea* (Solanaceae). Chemotaxonomical considerations

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ABSTRACT

Nine withanolides were isolated from the aerial parts of *Deprea bitteriana*, *Deprea cuyacensis*, and *Deprea zamorae*. *D. bitteriana* yielded two withaphysalins, *D. cuyacensis* gave two 13,14-seco withaphysalins, while *D. zamorae* yielded five physangulidines. The compounds were fully characterized by a combination of spectroscopic methods (1D and 2D NMR and MS). All compounds isolated from *D. bitteriana* and *D. cuyacensis* were obtained as epimeric mixtures at C-18. The structure of physangulidine D was confirmed by X-ray diffraction analysis. The skeletons found in this research support the chemotaxonomical position of the genus *Deprea* in the tribe Physalideae.

1. Introduction

The withanolides comprise a class of naturally occurring C28-steroidal lactones structurally based on the ergostane skeleton. These compounds are generally polyoxygenated, and this profusion of oxygen functions has led to several natural modifications of the carbocyclic skeleton, as well as of the side-chain, resulting in compounds with complex structural features. Their chemistry and occurrence has been the subject of several reviews (Ray and Gupta, 1994; Veleiro et al., 2005; Chen et al., 2011; Misico et al., 2011). Withanolides are present almost exclusively in the Solanaceae subfamily Solanoideae. Some clades of the subfam. Solanoideae also contain withanolides with exclusive interesting structural arrangements which can be considered at different hierarchical levels as chemotaxonomic markers (Misico et al., 2011).

Deprea Raf. is a small neotropical genus from South America comprising ten species (Cueva and Treviño, 2012; Barboza et al., 2013). In *Deprea* only two species have been investigated, *Deprea orinocensis* and *Deprea subtriflora*. A family of withajardins (withajardins A–E) has been isolated from *D. orinocensis* (Luis et al., 1994; Echeverri et al., 1995) and many of these compounds exhibited leishmanicidal activity (Cardona et al., 2006). This nucleus, characterized by the presence of a bicyclic side-chain involving C-21 and C-25 has been only described in *Deprea* and *Tubocapsicum* genera (Hsieh et al., 2007; Kiyota et al., 2008). Regarding *D. subtriflora* [currently, *Larnax subtriflora*, (Hunziker, 2001)], a biology-guided study led to isolation of a family of highly oxygenated C27 18-norwithanolides named subtrifloralactones (subtrifloralactones A–L). Some of these subtrifloralactones showed potential cancer chemopreventive activity (Su et al., 2003a,b).

Based on morphological data, Hunziker (2001) placed the genus in the tribe Solaneae subtribe Witheringiinae Reveal, while Sawyer (2005), based on a morphological phylogenetic analysis, considered *Deprea* to be a member of the tribe Physalideae Miers (Barboza et al., 2013). Recently, Särkinen et al. (2013), from molecular phylogenetic evidence, included *Deprea* in the tribe Physalideae, although the genus was not assigned to any subtribe. As part of an ongoing program here aimed at determining the content of withanolides contributing to the taxonomic position of *Deprea*, report in herein is the isolation of a set of both new and known withanolides from *Deprea bitteriana* (Werderm.) N.W. Sawyer & Benítez, *Deprea cuyacensis* (N.W. Sawyer & S. Leiva) S. Leiva & Lezama, and *Deprea zamorae* Barboza & S. Leiva.

2. Results and discussion

The dichloromethane extracts of the aerial parts of three *Deprea* species were subjected to chromatographic purification and

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yielded eight new withanolides (**1**–**8**, Fig. 1) and the known withaphysalin C.

2.1. Withanolides isolated from D. bitteriana

D. bitteriana afforded the new compounds withaphysalins V (**1**) and W (**2**). Withaphysalins are characterized by oxygenation at the Me(18) group to the level of an alcohol, aldehyde, or acid functioality, which in the latter two cases can lead to cyclization with OH-C(20) to yield a hemiacetal or a lactone. The hemiacetal arrangement is characterized by the absence of the methyl-18 ^{1}H NMR singlet at low-frequency shift, and by the appearance of two methine carbynolic resonances, indicating the presence of *R*/*S* mixtures of epimeric hemiacetals at C-18.

The HRESIMS of withaphysalin V (**1**) showed a molecular ion $[M+Na]^+$ at *m*/*z* 491.2383, corresponding to an elemental formula of $C_{28}H_{36}O_6Na$. Inspection of the 1D and 2D NMR spectroscopic data indicated that compound **1** possessed rings C and D, as well as the side-chain closely related to those of withaphysalins B [isolated for the first time from *Physalis minima* (Glotter et al., 1975)], F, and G [isolated for the first time from *Eriolarynx lorentzii* (Misico et al., 2000)]. The absence of a signal for CH_3-18 and the presence of two proton resonances at δ 5.18 (1H, *br s*) (50%) and 5.31 (1H, *br s*) (50%) (Table 1) were indicative of a lactol functionality at C-18 and were assigned to the 18*R* and 18*S* epimers, respectively. The ^{13}C NMR spectrum showed methine signals at δ 101.3 and 103.9 ppm (C-18 *R*/*S*) and resonances at δ 58.1/58.9, δ 56.8/56.8, and δ 84.9/85.2 assigned at C-13 *R*/*S*, C-17 *R*/*S*, and C-20 *R*/*S*, respectively, thus confirming a five-membered lactol ring (Table 3). The doublet of doublets at δ 4.46/4.54 (H-22 *R*/*S*), together with the two three-proton singlets at δ 1.88 (H_3-27) and 1.95 (H_3-28), indicated the presence of a δ lactone as a side-chain. The resonances at δ 80.6/80.4 (C-22 *R*/*S*), 148.0/148.8 (C-24 *R* and *S*), 121.9/122.2 (C-25 *R* and *S*), 165.6/165.7 (C-26 *R* and *S*), 12.4/12.5 (C-27 *R* and *S*), and 20.4/20.5 (C-28 *R* and *S*), observed in the ^{13}C NMR spectrum, were in agreement with a δ lactone. Regarding rings A and B, the ^{1}H NMR spectrum of the epimeric mixture showed characteristic signals of H-2, H-3, and H-6 hydrogens for a 1-oxo-2,5-withadienolide at δ 5.91 (*dd*, H-2), 6.81/6.80 (*ddd*, H-3), and 5.84/5.83 (*m*, H-6), respectively (Ramacciotti and Nicotra, 2007). The correlation found between H-6 with H-7 at δ 3.95 in the COSY spectrum suggested the hydroxy group to be at C-7. Olefinic resonances at δ 128.0, 144.9/144.6, 141.4/140.9, and 126.0/126.4, a carbonyl carbon at δ 203.0/203.3 (C-1 *R*/*S*), and a carbynolic carbon at δ 64.5 (C-7 *R*/*S*) were observed in the ^{13}C NMR spectrum. The α orientation of the hydroxy group at C-7 was established by a NOESY experiment. The NOE observed between H-7 and H-8 *R*/*S* (δ 1.51/1.92) indicated the β orientation of H-7. Accordingly, the structure of compound **1** was determined as shown.

The molecular formula of withaphysalin W (**2**) was determined by HRESIMS as $C_{28}H_{36}O_6$. Similar to **1**, compound **2** was demonstrated to be a 1:1 epimeric mixture from its ^{1}H NMR spectrum, which showed characteristic H-18 epimeric protons resonating at δ 5.21 *s* and 5.31 *s*. Their ^{1}H and ^{13}C NMR spectra were closely related to those of compound **1**, differing only in the substitution pattern of ring A (Tables 1 and 3). A 1-oxo-3,5-diene-7α-hydroxy substitution pattern was inferred from the following observations: (i) two mutually coupled olefinic signals resonating at δ 5.81 (*m*) and 6.10 (*br d*, *J* = 9.7 Hz) assigned to H-3 and H-4, respectively;

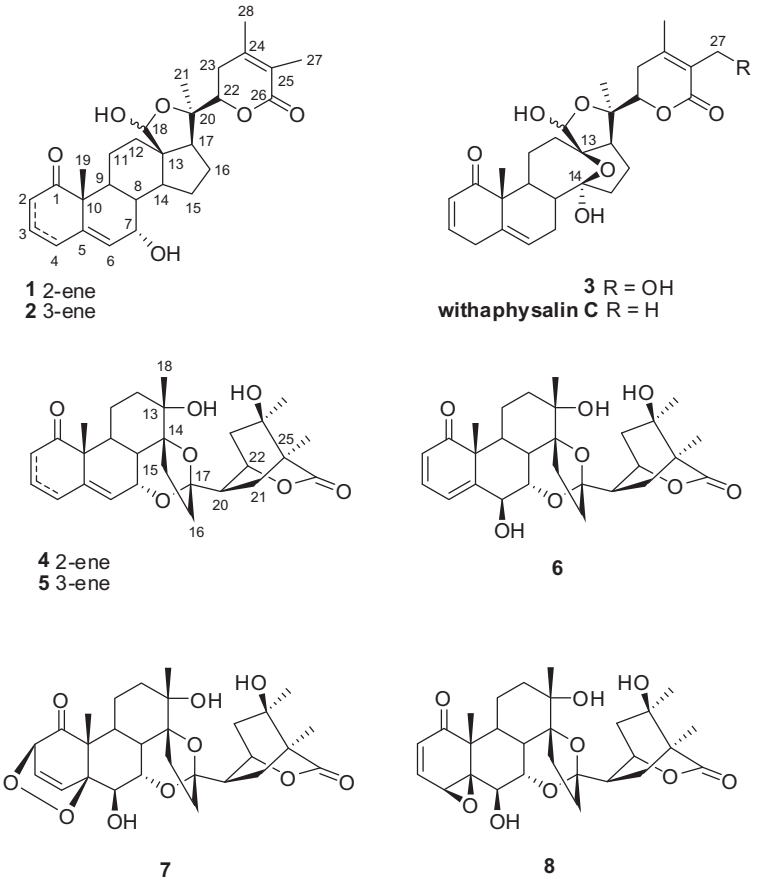

Fig. 1. Withanolides isolated from species of *Deprea*.

Table 1
^{1}H NMR spectroscopic data of compounds **1**–**3** in $CDCl_3$.[a]

Position	**1** 18*R* and 18*S*	**2** 18*R* and 18*S*	**3** 18*R*
2a	5.91 *dd* (9.6, 2.6)	3.32 *br d* (20.4)	5.88 *dd* (9.9, 1.7)
2b	–	2.79 *dd* (20.4, 4.6)	–
3	6.81 *ddd* (9.6, 4.8, 2.6) *R*/ 6.80 *ddd* (9.6, 4.7, 2.6) *S*	5.81 *m* –	6.76 *ddd* (9.9, 5.0, 2.5) –
4α	2.90 *dd* (21.6, 4.8)	6.10 *br d* (9.7)	2.85 *dd* (21.0, 5.0)
4β	3.35 *br d* (21.6)	–	3.29 *br d* (21.0)
6	5.84 *m R*/5.83 *m S*	5.82 *m*	5.62 *br d* (5.2)
7a	3.95 *m*	4.05 *m*	2.27 *m*
7b	–	–	1.78 *m*
8	1.51 *m R*/1.92 *m S*	1.62 *m R*/2.01 *m S*	1.90 *m*
9	2.02 *m R*/2.05 *m S*	2.11 *m R*/2.14 *m S*	1.90 *m*
11a	–	–	2.07 *m*
11b	–	–	1.48 *m*
12a	2.58 *br d* (12.4) *R*/2.04 *m S*	2.55 *m R*/1.99 *m S*	2.15 *m*
12b	1.64 *m R*/1.54 *m S*	1.61 *m R*/1.53 *m S*	2.00 *m*
14	1.74 *m R*/1.84 *m S*	1.76 *m R*/1.86 *m S*	–
15a	–	–	1.93 *m*
15b	–	–	1.71 *m*
16a	1.89 *m R*/1.93 *m S*	1.92 *m S*	1.87 *m*
16b	1.76 *m R*/1.82 *m S*	1.85 *m S*	–
17	2.12 *m*	2.11 *m R*/2.08 *m S*	2.08 *m*
18	5.18 *br s R*/5.31 *br s S*	5.21 *s R*/5.31 *s S*	5.11 *d* (7.8)
19	1.21 *s R*/1.23 *s S*	1.32 *s R*/1.34 *s S*	1.25 *s*
21	1.51 *s R*/1.31 *s S*	1.50 *s R*/1.31 *s S*	1.28 *s*
22	4.46 *dd* (13.2, 3.3) *R*/ 4.54 *dd* (13.2, 3.3) *S*	4.45 *dd* (13.2, 3.2) *R*/ 4.57 *dd* (13.2, 3.0) *S*	4.35 *m* –
23a	2.42 *m R*/2.52 *m S*	2.40 *m R*/2.48 *m S*	3.04 *dd* (18.1, 13.1)
23b	2.05 *m R*/2.18 *m S*	2.04 *m R*/2.17 *m S*	2.24 *m*
27a	1.88 *s*	1.87 *s*	4.40 *m*
27b	–	–	4.33 *m*
28	1.95 *s*	1.95 *s*	2.07 *s*
OH-18	–	–	4.45 *m*

[a] Chemicals shifts (δ) downfield from TMS, *J* couplings (in parentheses) in Hz. 400.13 MHz.

(ii) the downfield shift of the carbonyl carbon in the ^{13}C NMR spectrum from δ 203.0/203.3 for compound **1** to δ 209.6/209.8 for compound **2**, assigned to C-1; (iii) the downfield shift of H_3-19 in the ^{1}H NMR spectrum from δ 1.21/1.23 for **1** to δ 1.32/1.34 for **2**. The ^{13}C NMR signals corresponding to the A and B rings of withaphysalin W were in agreement with the proposed structure (García et al., 2012).

2.2. Withanolides isolated from D. cuyacensis

Following a similar procedure, the EtOH extract of *D. cuyacensis* was analyzed and two 13,14-seco withaphysalins were isolated, namely the previously determined withaphysalin C isolated from *Physalis minima* (Kirson et al., 1976) and compound **3**, named withaphysalin X. Compound **3** had a molecular formula of $C_{28}H_{36}O_8$ by HRESIMS. Inspection of the 1D and 2D NMR spectroscopic data indicated that compound **3** possessed a 13,14-seco-ergostane nucleus closely related to that of withaphysalin C (Kirson et al., 1976) and its 18-acetyl derivative (Ma et al., 2007) (Tables 1 and 3). Compound **3** (C-18*R*) was characterized from a mixture of epimers C-18*R*/C-18*S* in a 2:1 ratio, and was found to contain the following moieties in common with withaphysalin C: a 1-oxo-2,5-diene system in rings A/B, an hemiacetal arrangement involving C-18 and C-20 positions, a 13β,14β-epoxy function, and a δ-lactone in the side chain, in agreement with the ^{1}H–^{1}H COSY, HSQC, and HMBC experiments. The only difference observed between **3** and withaphysalin C was the missing C-27 methyl signal and the resonance at δ 4.40 *m* and 4.33 *m*, assigned to H-27a and H-27b, respectively, indicating that C-27 was oxidized to a hydroxymethyl group. This was consistent with the methylene carbon resonance at δ 56.8 in the ^{13}C NMR and DEPT spectra.

2.3. Withanolides isolated from Deprea zamorae

Finally, the EtOH extract of *D. zamorae* yielded five new withanolides, named physangulidines DH (**48**). This unusual nucleus is characterized by the typical arrangement of withajardins, where C-21 is directly bonded to C-25 resulting in a bicyclic lactone side-chain with a six-membered homocycle. Regarding the steroid nucleus, physangulidines have a ketal functionality at the C-17 position formed through oxidative cleavage of the C13—C17 bond and subsequent nucleophilic attack of the C-14 and C-7 hydroxy groups (Fig. 2). Compound **4**, $C_{28}H_{36}O_7Na$, showed a peak at *m*/*z* 507.2352, corresponding to [M+Na] in the HRESIMS mass spectrum. The ^{1}H and ^{13}CNMR spectra of withanolide **4** closely resembled those of physangulidine C, recently isolated from *Physalis angulata* (Zhuang et al., 2012). Characteristic signals assigned to the side-chain and the resonances corresponding to the ketal functionality involving the C17—C7 and C17—C14 positions were observed (Tables 2 and 3). Regarding the rings A and B substitution patterns, the ^{1}H and ^{13}C NMR spectra of **4** had almost identical signals for all carbons and protons of rings A and B of compound **1**, indicating a 1-oxo-2,5-diene-7α,O-substitution. Confirmation of the structure of **4** and assignment of the configuration at C-7, C-13, C-14, and C-17 were obtained from X-ray diffraction analysis. The diffraction data afforded the structure depicted in Fig. 3, where the orientation for the hydroxy group at position 13 was established as α. The *R* configuration for C-20, C-22, C-24, and C-25 was also evident.

The ^{1}H and ^{13}C NMR spectra of withanolides **5**–**8** (Tables 2 and 3) were closely related to those of **4,** showing patterns typical of the physalngulidins. The almost identical ^{13}C NMR spectroscopic data for rings C, D and the side-chain of compounds **4**–**8** indicated

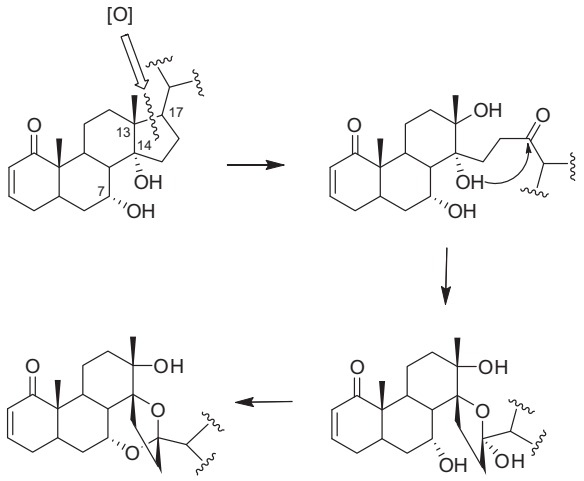

Fig. 2. Physangulidine proposed biosynthetic route.

that structural differences were restricted to substituents in rings A and B. The NMR spectroscopic data of compound **5** suggested a 1-oxo-3,5-diene system in rings A and B. This assumption was confirmed by comparison of the ^{1}H and ^{13}C NMR data of **5** and compound **2** (Tables 1–3).

The ^{1}H NMR spectrum of **6** (Table 2) displayed signals at δ 6.12 (*d*, *J* = 9.7 Hz), 7.00 (*dd*, *J* = 9.7, 6.0 Hz) and 6.25 (*d*, *J* = 6.0 Hz), assigned to three olefinic hydrogens at C-2, C-3 and C-4, respectively. In addition to these resonances, the ^{1}H NMR spectrum displayed a carbynolic hydrogen signal at δ 4.34 (*d*, *J* = 2.3 Hz) assigned to H-6. The presence of the 1-oxo-2,4-diene-6β-hydroxy moiety was confirmed by the resonances at δ 203.8, 127.3, 139.4, 121.3, 152.7, 76.3, and 74.0 in the ^{13}C NMR spectrum, assigned to C1–C7, respectively. The β orientation of hydroxy group at C-6 was established by the strong intensity NOE observed between H-6 with H-4.

Regarding the A/B rings of compound **7**, the ^{1}H and ^{13}C NMR spectra of **7** (Table 2) exhibited a close resemblance to those of physalin Q isolated from *Physalis alkekengi* var. *francheti* (Makina et al., 1995) possessing the same 1-oxo-3-ene-2β,5β-epidioxy-6β-hydroxy substitution pattern. The ^{1}H NMR spectrum of **7** showed signals for the two coupled olefinic protons at δ 6.63 (*dd*, *J* = 8.3, 6.5 Hz) and 6.80 (*dd*, *J* = 8.3, 1.4 Hz), assigned to the H-3 and H-4 vicinal protons, respectively. In addition to these resonances, the ^{1}H NMR spectrum displayed a carbynolic hydrogen signal at δ 4.63 (*dd*, *J* = 6.5, 1.5 Hz) which showed connectivity in the COSY spectrum with the hydrogen resonance at δ 6.80, indicating the hydroxy group at C-2. The signal at δ_H 4.12 (*dd*, *J* = 2.2, 1.5 Hz) indicated that the compound **7** also possessed hydroxyl substituent located at C-6, supported by HMBC correlations from H-4 to C-6. The ^{13}C NMR spectrum of **7** was in agreement with the structure proposed for the resonances at δ 204.7, 80.3, 124.8, 142.4, 83.0, and 70.8 assigned to C1C6, respectively. The β orientation of the C-2/C-5 peroxy bridge was established by the NOE observed between the H-4 and H-22 (δ 4.73) signals, indicating the *cis* rings A/B fusion (see Supporting Information), while the β orientation of the hydroxy group at C-6 was established by a cross-correlation peak observed between the H-6 and H-4 resonances in the NOESY experiment.

Finally, the ^{1}H NMR spectrum of **8** (Table 2) showed characteristic chemical shifts for the 1-oxo-2-ene-4β,5β-epoxy system at ring A, with signals for H-2 and H-3 being clearly distinguished at δ 6.09 (*dd*, *J* = 9.9, 1.5 Hz) and δ 7.03 (*dd*, *J* = 9.9, 4.3 Hz), respectively. The correlation observed in the COSY experiment between the pair H-3/H-4 led to the assignment of H-4 at δ 3.37 (*dd*, *J* = 4.4, 1.4 Hz) of the 4β,5β-epoxy function. *Cis* rings A/B fusion was established from a NOESY experiment, where the NOE correlation observed for H-4/H-22 (δ 4.64) indicated the presence of an epoxy group with a β-orientation (see Supporting Information). The ^{1}H NMR spectrum of **8** exhibited a signal at δ 3.45 assigned to a carbynolic proton at C-6 indicating a hydroxy group. The cross-correlation peak between the H-6 and H-4 resonances in the NOESY experiment indicated the β orientation of the hydroxy group at C-6. The ^{13}C NMR spectrum showed the expected chemical shifts for signals

Table 2
^{1}H NMR spectroscopic data of compounds **4**–**8** in $CDCl_3$.[a]

Position	**4**	**5**	**6**	**7**	**8**
2a	5.94 *dd* (10.0, 2.6)	3.33 *dt* (20.4, 3.0)	6.12 *d* (9.7)	4.63 *dd* (6.5, 1.4)	6.09 *dd* (9.9, 1.5)
2b	–	2.84 *ddd* (20.4, 4.8, 1.0)	–	–	–
3a	6.80 *ddd* (10.0, 4.9, 2.6)	5.87 *ddd* (9.7, 4.8, 3.0)	7.00 *dd* (9.7, 6.0)	6.63 *dd* (8.3, 6.5)	7.03 *dd* (9.9, 4.3)
4α	3.00 *dd* (21.4, 4.9)	6.13 *dd* (9.7, 2.5)	6.25 *d* (6.0)	6.80 *dd* (8.3, 1.4)	3.37 *dd* (4.4, 1.5)
4β	3.31 *br d* (21.4)	–	–	–	–
6	5.62 *dd* (5.6, 1.8)	5.61 *d* (5.4)	4.34 *d* (2.3)	4.12 *dd* (2.2, 1.5)	3.45 *br s*
7	4.22 *t* (3.8)	4.33 *t* (4.5)	4.14 *t* (2.5)	4.24 *t* (2.3)	4.20 *t* (2.7)
8	1.37 *dd* (12.5, 3.8)	1.44 *dd* (12.6, 4.0)	1.96 *m*	1.82 *m*	1.81 *dd* (11.6, 3.2)
9	2.30 *td* (12.5, 4.1)	2.44 *m*	1.91 *m*	1.85 *m*	2.08 *m*
11a	2.47 *m*	2.03 *m*	2.06 *m*	2.43 *m*	2.06 *m*
11b	1.30 *m*	1.15 *m*	1.29 *m*	1.43 *m*	1.28 *m*
12a	1.93 *td* (13.0, 3.5)	1.93 *m*	1.76 *m*	1.73 *m*	1.72 *m*
12b	1.73 *dt* (13.0, 3.3)	1.71 *m*	1.56 *m*	1.66 *m*	1.60 *m*
15a	2.37 *td* (12.8, 5.2)	2.38 *td* (12.9, 4.8)	2.36 *m*	2.33 *m*	2.35 *m*
15b	1.67 *ddd* (14.0, 9.5, 4.9)	1.70 *m*	1.76 *m*	1.73 *m*	1.75 *m*
16a	2.02 *m*	2.03 *m*	2.02 *m*	2.05 *m*	2.05 *m*
16b	1.98 *m*	2.03 *m*	1.98 *m*	2.02 *m*	2.01 *m*
18	1.20 *s*	1.20 *s*	1.23 *s*	1.22 *s*	1.21 *s*
19	1.20 s	1.29 *s*	1.41 *s*	1.50 *s*	1.45 *s*
20	2.51 *m*	2.52 *m*	2.51 *m*	2.61 *m*	2.56 *m*
21a	2.25 *dd* (14.1, 6.0)	2.24 *dd* (14.2, 6.1)	2.12 *dd* (14.3, 6.2)	2.16 *dd* (13.9, 6.5)	2.16 *dd* (14.0, 6.5)
21b	1.56 *dd* (14.1, 11.9)	1.57 *dd* (14.2, 11.8)	1.55 *m*	1.56 *m*	1.57 *dd* (14.0, 11.7)
22	4.70 *td* (3.6, 1.7)	4.70 *td* (3.6, 1.8)	4.61 *td* (3.3, 1.6)	4.73 *td* (3.6, 1.4)	4.64 *td* (3.6, 1.6)
23a	2.44 *dd* (15.5, 1.7)	2.46 *dd* (15.5, 1.8)	2.25 *dd* (15.4, 1.6)	2.00 *m*	2.37 *dd* (15.4, 1.8)
23b	2.01 *m*	2.02 *m*	1.96 *m*	2.04 *m*	1.99 *m*
27	1.15 *s*	1.15 *s*	1.12 *s*	1.16 *s*	1.16 *s*
28	1.18 *s*	1.19 *s*	1.17 *s*	1.21 *s*	1.20 *s*

[a] Chemicals shifts (δ) downfield from TMS, *J* couplings (in parentheses) in Hz. 400.13 MHz.

Table 3
^{13}C NMR spectroscopic data of compounds **18** in $CDCl_3$.[a]

Position	**1** 18*R* and 18*S*	**2** 18*R* and 18*S*	**3** 18*R*	**4**	**5**	**6**	**7**	**8**
1	203.0 *R*/203.3 *S*	209.6 *R*/209.8 *S*	204.1	202.2	208.3	203.8	204.7	198.1
2	128.0	39.5	127.7	128.0	39.4	127.3	80.3	130.7
3	144.9 *R*/144.6 *S*	125.2/125.5	144.9	144.5	126.1	139.4	124.8	140.7
4	33.3	129.0/129.1	33.2	32.8	128.5	121.3	142.4	54.1
5	141.4 *R*/140.9 *S*	144.6 *R*/144.3 *S*	135.1	143.4	146.0	152.7	83.0	63.7
6	126.4 *R*/126.0 *S*	126.8/127.3	125.1	121.7	122.5	76.3	70.8	75.1
7	64.5	64.7	26.7	64.9	65.3	74.0	73.0	73.0
8	38.9 *R*/37.9 *S*	37.8 *R*/36.8 *S*	47.5	39.3	38.7	38.4	37.8	37.2
9	34.7 *R*/35.2 *S*	33.3 *R*/33.7 *S*	43.2	32.7	31.4	39.6	40.1	37.4
10	51.2 *R*/51.1 *S*	52.8	52.7	50.6	52.4	52.8	47.7	49.4
11	25.4	24.0/24.3	25.8	24.7	23.5	21.4	24.1	23.1
12	34.6 *R*/37.0 *S*	34.4 *R*/36.8 *S*	43.2	37.6	37.4	37.9	37.8	37.9
13	58.1 *R*/58.9 *S*	58.2 *R*/59.1 *S*	79.1	71.1	71.3	71.0	71.0	71.0
14	50.6 *R*/49.0 *S*	50.2 *R*/48.5 *S*	101.1	88.1	87.7	89.0	89.0	88.9
15	25.7 *R*/25.2 *S*	25.3/25.2	27.4	27.7	27.8	27.5	27.3	27.2
16	25.9 *R*/27.2 *S*	25.9 *R*/27.2 *S*	15.0	33.3	33.3	33.7	33.2	33.5
17	56.8 *R*/56.8 *S*	56.9 *R*/56.7 *S*	55.0	107.1	107.2	108.0	108.4	108.2
18	101.3 *R*/103.9 *S*	101.3 *R*/103.7 *S*	106.1	21.8	21.9	22.1	22.4	22.1
19	18.3 *R*/18.4 *S*	19.5 *R*/19.6 *S*	18.2	18.2	19.3	17.3	15.4	13.1
20	84.9 *R*/85.2 *S*	84.8 *R*/85.2 *S*	85.0	41.9	42.0	41.9	41.1	41.6
21	21.7 *R*/24.7 *S*	21.7 *R*/24.4 *S*	25.8	26.6	26.7	26.4	26.7	26.2
22	80.6 *R*/80.4 *S*	80.6 *R*/80.6 *S*	78.0	74.6	74.7	74.5	74.6	74.4
23	31.2 *R*/32.0 *S*	31.2 *R*/32.0 *S*	31.1	40.8	40.9	40.8	40.4	40.7
24	148.0 *R*/148.8 *S*	148.2 *R*/148.9 *S*	155.3	70.7	70.8	70.7	70.9	71.2
25	121.9 *R*/122.2 *S*	122.1 *R*/121.9 *S*	125.1	47.6	47.7	47.5	47.7	47.6
26	165.6/165.7	165.9	166.5	177.8	177.8	177.3	177.2	177.1
27	12.4/12.5	12.4	56.8	14.2	14.3	14.2	14.3	14.3
28	20.4/20.5	20.4	20.1	27.9	27.9	27.8	28.3	28.1

[a] Chemical shifts (δ) downfield from TMS; 100.03 MHz.

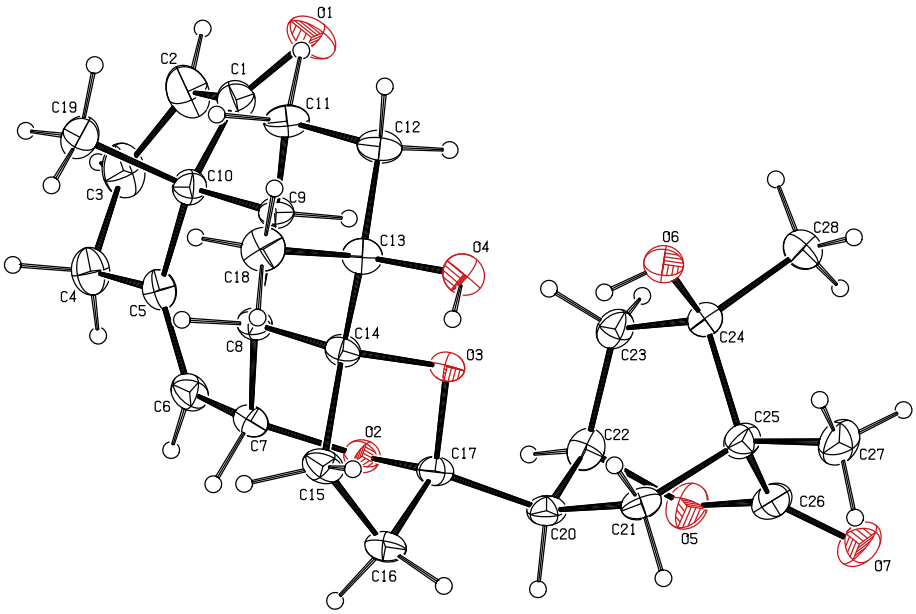

Fig. 3. ORTEP diagram of physangulidine D (**4**) showing one molecule of the asymmetric unit with the atom-numbering scheme. Displacement ellipsoids are drawn at the 30% probability level and H atoms are shown as small spheres of arbitrary radii.

corresponding to rings A/B carbons at δ 198.1 (C-1), 130.7 (CH-2), 140.7 (CH-3), 54.1 (CH-4), 63.7 (C-5), 75.1 (CH-6), and 73.0 (CH-7), respectively.

The full and unambiguous proton and carbon NMR assignments for compounds **5**–**8** were confirmed using a combination of DEPT-135, COSY, HSQC, HMBC, and NOESY experiments. Moreover, the high-resolution mass measurements were in agreement with the proposed formulas.

2.4. Chemotaxonomical considerations

Recently, *Deprea* has been suggested to be placed within the tribe Physalideae, based on molecular evidence of only three studied species (Särkinen et al., 2013). The results herein support this hypothesis, since the withanolides isolated in the *Deprea* species analyzed here (withaphysalins, withajardins, and physangulidines) are exclusive to this tribe.

Within *Deprea, D. bitteriana* and *D. cuyacensis* possess only withaphysalins. This skeleton is exclusive to subtribe Physalinae and Lochrominae so far (Misico et al., 2011). To date, at least twenty withaphysalins have been reported from the *Acnistus*, *Dunalia*, *Eriolarynx*, and *Physalis* genera. It should also be mentioned that whitaphysalins are biogenetic precursors of the physalins that have only been isolated in the genera belonging to the subtribe Physalinae (Misico et al., 2011). The other species studied, *D. zamorae*, contains five physangulidines, a nucleus recently described from *Physalis angulata* (Zhuang et al., 2012), which has the same side-chain as the withajardins previously isolated from *D. orinocensis* (Luis et al., 1994; Echeverri et al., 1995). Moreover, physangulidine G (**7**) has a 1-oxo-3-ene-2β,5β-epidioxy substitution pattern in ring A, an unusual structural arrangement that has only been described in physalin Q isolated from *Physalis alkekengi* var. *francheti* (Makina et al., 1995).

As only four species out of a total of ten have been investigated from a chemical point of view, the available data are still not conclusive to suggest a possible inclusion of *Deprea* in any subtribe within Physalideae.

3. Conclusions

The phytochemical information here provided is in agreement with the arrangement of the metabolites that have been isolated in many genera of the tribe Physalideae, thus supporting the inclusion of the genus *Deprea* in this tribe but not in a particular subtribe. The phytochemical studies and the molecular analysis of the remaining species of *Deprea* that are being carried out by our group – with a more comprehensive and detailed molecular analysis than that previously performed by Olmstead et al. (2008) or Särkinen et al. (2013) – will provide more conclusive results regarding the position of *Deprea* at subtribal level.

4. Experimental

4.1. General

Melting points were measured on a mercury thermometer apparatus and were uncorrected, whereas optical rotations were recorded using a JASCO P-1010 polarimeter. The UV spectra and IR spectra were obtained using a Shimadzu-260 spectrophotometer and Nicolet 5-SXC spectrophotometer, respectively. NMR experiments were performed on a Bruker AVANCE II 400 MHz instrument. Multiplicity determinations (DEPT) and 2D spectra (COSY, HSQC, HMBC, and NOESY) were obtained using standard Bruker software. Chemical shifts are given in ppm (δ) downfield from TMS internal standard. HRESIQTOFMS were measured on a Micro TOFQ II Bruker Daltonics (MA, USA). Single crystal X-ray measurement was performed on Oxford Diffraction CCD area detector diffractometer. All H atoms were refined as riding on their parent atoms, with U_{iso}(H) values set at 1.2 Ueq or 1.5U_{eq} of the parent C atoms. The EtOH solvent molecule was found to show unresolvable disorder and its contribution to the scattering was removed with the SQUEEZE option in *PLATON* (Spek, 2009).

Chromatographic separations were performed by column chromatography (CC) on silica gel 60 (0.063–0.200 mm) and Sephadex LH-20 (Sigma–Aldrich), radial chromatography was carried out employing a radial Chromatotron Model 7924 T on silica gel 60 PF_{254} Merck (1 mm thick), and prep. TLC was performed on silica gel 60 F_{254} (0.2 mm thick) plates.

4.2. Plant material

Voucher specimens of three species are deposited at CORD (Córdoba, Argentina) herbarium. *D. bitteriana* was collected in Cundinamarca Department, Colombia, in August 2011 (Barboza et al. *3054 bis*); *D. cuyacensis* in Piura Department, Perú, in January 2012 (Barboza et al. 3367), and *D. zamorae* in Zamora Chinchipe Province, Ecuador, in November 2011 (Barboza et al. 3083 bis). In all cases, a flowering and fruiting branch was collected. The species were identified by Gloria E. Barboza (IMBIV-CONICET, Córdoba, Argentina).

4.3. Extraction and isolation of compounds from Deprea species

The dried and pulverized aerial parts of *D. bitteriana* (162.6 g) were exhaustively extracted using EtOH at room temperature, and the solvent was evaporated under reduced pressure. The resulting residue (14.5 g) was suspended in EtOH–H_2O (3:1) and washed with *n*-hexane (5 × 400 mL), after which the EtOH was evaporated under reduced pressure. The residue was diluted with H_2O and extracted with CH_2Cl_2 (5 × 300 mL). The combined CH_2Cl_2 phases were dried (Na_2SO_4), filtered, and evaporated to dryness under reduced pressure. Next, the organic residue obtained by hexane partition (6.5 g) was fractionated by Sephadex LH-20 CC, using a hexane/$CHCl_3$/MeOH (2:1:1) mixture for the elution, to afford a mixture of two closely related compounds subsequently identified as epimers of compound **1** (107 mg). The residue obtained by CH_2Cl_2 partition (4.4 g) was fractionated initially by exclusion Sephadex LH-20 CC. Elution with MeOH afforded three fractions, two of which contained withanolides. Fraction II (3.3 g) was applied to a silica gel 60 G column, using CH_2Cl_2:MeOH mixtures of increasing polarity (100:0–90:10) for the elution, to afford to eight fractions. Fraction 1 (50 mg) was further purified by preparative TLC with CH_2Cl_2/MeOH, 97.5:2.5, to give mixture of epimers compound **1** (8 mg). Fraction 2 (2.0 g), was subjected to silica gel 60 G CC. Elution with CH_2Cl_2:MeOH (100:0–95:05) afforded 8 subfractions. Of these, subfraction 2 (105 mg) was further fractionated by radial chromatography (CH_2Cl_2–MeOH 98:02), yielding compound **1** (18 mg), while subfraction 3 (330 mg) was fractionated by radial chromatography (CH_2Cl_2–MeOH 97:03) to afford an epimeric mixture of compound **2** (110 mg).

The air-dried powdered aerial parts of *D. cuyacensis* (63 g) were exhaustively extracted with EtOH, with the solvent being evaporated at reduced pressure. The residue obtained (7.8 g) was defatted by partitioning in *n*-hexane–EtOH–H_2O (10:3:1), with the resulting EtOH–H_2O phase washed with *n*-hexane (3 × 200 mL), and the EtOH evaporated under reduced pressure. The residue was diluted with H_2O and extracted with CH_2Cl_2 (3 × 200 mL). The CH_2Cl_2 extract was dried (Na_2SO_4), filtered, and evaporated to dryness under reduced pressure. This resulting residue (3.1 g) was initially fractionated by Sephadex LH-20 CC. Elution with MeOH afforded two fractions, one of which contained withanolides. This fraction (II, 2.1 g) was subjected to silica gel 60 G CC. Elution with CH_2Cl_2:MeOH mixtures of increasing polarity (100:0–90:10) afforded six fractions. Of these, fraction 1 was an epimeric mixture of withaphysalin **C** (420 mg), whereas fraction 5 (590 mg) was fractionated by silica gel CC using CH_2Cl_2:MeOH mixtures of increasing polarity (100:0–95:05) to give 495 mg of a binary mixture of closely related compounds, identified as an epimeric mixture of compound **3**.

The air-dried powdered aerial parts of *D. zamorae* (58 g) were extracted with EtOH at room temperature, the solvent was evaporated, and the residue (5.2 g) was partitioned between *n*-hexane, EtOH, and H_2O (30:3:1). Then, the aqueous EtOH layer was washed with hexane (5 × 250 mL), concentrated, and extracted with CH_2Cl_2 (4 × 250 mL). The residue (2.6 g), obtained after evaporation of the solvent, was initially subjected to Sephadex LH-20 exclusion CC. Elution with MeOH afforded three fractions, one of which contained withanolide (II, 2.3 g). This fraction was again applied to a Sephadex column using a hexane/$CHCl_3$/MeOH (2:1:1) mixture for the

elution. A portion (150 mg) of fraction 2 (1.0 g) was processed by radial chromatography to give compound **4** (19.2 mg) and an impure fraction (120 mg), with the latter being further fractionated by TLC (CH_2Cl_2–MeOH 97.5:2.5), yielding of compound **5** (14 mg). Fraction 3 (390 mg) was applied to a silica gel 60 G column using CH_2Cl_2:MeOH mixtures of increasing polarity (100:0–93:07) to afford thirteen subfractions. The subfraction 6 (13.3 mg) was fractionated by TLC with CH_2Cl_2–MeOH (96:04) to yield compound **7** (1.4 mg) and subfraction 8 (16.0 mg) was fractionated by TLC with CH_2Cl_2–MeOH (96:04), yielding compound **8** (7.3 mg). Subfractions 9 (32.2 mg) and 10 (56.4 mg) were fractionated by TLC with CH_2Cl_2–MeOH, 96:04, yielding compound **6** (in amounts of 16.2 and 17.0 mg, respectively).

4.3.1. Withaphysalin V (1, 18R and 18S)

White amorphous powder; $[\alpha]_D^{21}$ +22.8 (c 0.48, acetone); UV (CH_3OH) λ_{max} (log ε) 222 (3.99) nm; IR (dry film) ν_{max} 3427, 2977, 2939, 2879, 1682, 1654, 1454, 1375, 1321, 1138, 761 cm^{-1}; For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 3; HRESIMS *m/z* 491.2383 $[M+Na]^+$ (calcd. for $C_{28}H_{36}O_6Na$, 491.2404).

4.3.2. Withaphysalin W (2, 18R and 18S)

White amorphous powder; $[\alpha]_D^{21}$ +26.9 (c 0.69, acetone); UV (CH_3OH) λ_{max} (log ε) 214 (3.48) nm; IR (dry film) ν_{max} 3427, 2939, 2879, 1714, 1685, 1451, 1394, 1378, 1312, 1135, 751 cm^{-1}; For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 3; HRESIMS *m/z* 491.2390 $[M+Na]^+$ (calcd. for $C_{28}H_{36}O_6Na$, 491.2404).

4.3.3. Withaphysalin X (**3**)

White amorphous powder; $[\alpha]_D^{21}$ −2.9 (c 2.0, acetone); UV (CH_3OH) λ_{max} (log ε) 244 (3.18) nm; IR (dry film) ν_{max} 3497, 2980, 2936, 1698, 1654, 1572, 1385, 1328, 1245, 1176, 916, 751 cm^{-1}; For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 3; HRESIMS *m/z* 523.2299 $[M+Na]^+$ (calcd. for $C_{28}H_{36}O_8Na$, 523.2302).

4.3.4. Physangulidine D (**4**)

Colorless crystals (hexane–CH_2Cl_2), mp 190–191 °C; $[\alpha]_D^{21}$ −49.9 (c 0.45, acetone); UV (CH_3OH) λ_{max} (log ε) 208 (3.74) nm; IR (dry film) ν_{max} 3465, 2977, 2933, 1739, 1663, 1467, 1378, 1195, 1147, 910, 751 cm^{-1}; For ^1H and ^{13}C NMR spectroscopic data, see Tables 2 and 3; HRESIMS *m/z* 507.2352 $[M+Na]^+$ (calcd. for $C_{28}H_{36}O_7Na$, 507.2353).

Crystallographic Data and Data Collection Parameters: Crystals of compound **4**, $C_{28}H_{36}O_7$: colorless prism, 0.38 × 0.20 × 0.09 mm^3, orthorhombic, space group $P2_12_12_1$, a = 11.87761(23)Å, b = 17.93818(37)Å, c = 24.65501(48)Å, $\alpha = \beta = \gamma = 90°$, V = 5527.34(18)$Å^3$, Dcalc = 1.224 Mg/m^3, Z = 8. For 9360 reflections $I > 2\sigma(I)$ [R(int) = 0.027] the final anisotropic full matrix least-squares refinement on F^2 for 643 variables converged at R_1 = 0.0394 and wR_2 = 0.0439 with S of 1.03 and Flack parameter of 1.061(147). Crystallographic data for the structure reported in this paper has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 991474. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax:+44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

4.3.5. Physangulidine E (**5**)

White amorphous powder; $[\alpha]_D^{21}$ −39.6 (c 0.89 acetone); UV (CH_3OH) λ_{max} (log ε) 224 (3.67) nm; IR (dry film) ν_{max} 3465, 2980, 2942, 1739, 1716, 1377, 1194, 1144, 750 cm^{-1}; For ^1H and ^{13}C NMR spectroscopic data, see Tables 2 and 3; HRESIMS *m/z* 507.2354 $[M+Na]^+$ (calcd. for $C_{28}H_{36}O_7Na$, 507.2353).

4.3.6. Physangulidine F (**6**)

White amorphous powder; $[\alpha]_D^{21}$ −167.5 (c 0.49, acetone); UV (CH_3OH) λ_{max} (log ε) 311 (3.50) nm, 205 (3.62) nm; IR (dry film) ν_{max} 3462 (s), 2984, 2936, 1739, 1654, 1616, 1366, 1198, 1150, 1059, 755 cm^{-1}; For ^1H and ^{13}C NMR spectroscopic data, see Tables 2 and 3; HRESIMS *m/z* 523.2302 $[M+Na]^+$ (calcd. for $C_{28}H_{36}O_8Na$, 523.2302).

4.3.7. Physangulidine G (**7**)

White amorphous powder; $[\alpha]_D^{21}$ +50.4 (c 0.09, acetone); UV (CH_3OH) λ_{max} (log ε) 207 (3.55) nm; IR (dry film) ν_{max} 3446, 2974, 2930, 1733, 1451, 1369, 1369, 1195, 1093, 907, 751 cm^{-1}; For ^1H and ^{13}C NMR spectroscopic data, see Tables 2 and 3; HRESIMS *m/z* 555.2204 $[M+Na]^+$ (calcd. for $C_{28}H_{36}O_{10}Na$, 555.2201).

4.3.8. Physangulidine H (**8**)

White amorphous powder; $[\alpha]_D^{21}$ −32.4 (c 0.37, acetone); UV (CH_3OH) λ_{max} (log ε) 230 (3.38) nm, 205 (3.44) nm; IR (dry film) ν_{max} 3449, 2980, 2939, 1730, 1676, 1375, 1147, 1081, 992, 903, 755 cm^{-1}; For ^1H and ^{13}C NMR spectroscopic data, see Tables 2 and 3; HRESIMS *m/z* 539.2251$[M+Na]^+$ (calcd. for $C_{28}H_{36}O_9Na$, 539.2252).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytochem.2014.11.015.

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